Discontinuation of Antiretroviral Therapy Commenced Early during the Course of Human Immunodeficiency Virus Type 1 Infection, with or without Adjunctive Vaccination

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Sixteen subjects were treated with highly active antiretroviral therapy within 120 days of the onset of symptoms of newly acquired human immunodeficiency virus type 1 (HIV-1) infection. Eleven of the 16 participated in an adjunctive therapeutic vaccine trial. After a mean of 3.2 years of treatment, they elected to discontinue therapy. Virus rebound occurred in all subjects and was followed by a spontaneous, transient although significant reduction in log plasma HIV-1 RNA level, ranging from 0.3 to 3.1 $\log_{10}$ copies/mL. Despite evidence of the induction of HIV-1–specific cell-mediated immune responses, plasma viremia was not persistently suppressed to <500 copies/mL in any subject. The magnitude and dynamics of virus rebound were similar in both vaccinated and unvaccinated subjects. Nevertheless, given the transient suppression of viremia observed in nearly all subjects after treatment has been discontinued, further investigations of adjunctive vaccination with optimized antiretroviral therapy in treating HIV-1 infection are warranted.

Highly active antiretroviral therapy (HAART) has durably suppressed viral replication in vivo, translating into significant clinical benefit [1]. In 1995, having observed unprecedented success in achieving a rapid suppression of virus replication in vivo with combination nucleoside reverse-transcriptase inhibitors and protease-inhibitor monotherapy, we embarked on a program to identify and treat subjects within ~90 days of the onset of symptoms of new infection with human immunodeficiency virus type 1 (HIV-1). We hypothesized that these subjects would most likely benefit from early intervention, on the basis of observations that the virus population during and shortly after transmission is homogeneous and, therefore, most likely to be susceptible to combination therapy [2]. Furthermore, given the short duration of infection, we theorized that the newly infected subject's immune system should be minimally impaired. Elsewhere, we have described virologic and immunologic details of the initial 12 subjects enrolled in this program [3].

Our early observations of a 2-phase decrease in HIV-1 RNA levels in plasma formed the foundation for the "eradication hypothesis" [4, 5]. On the basis of the decay rates of 2 identified HIV-1–infected cell populations, it was proposed that 2–3 years of complete suppression of viral replication could result in the eradication of the virus from these compartments. It was emphasized, however, that, for eradication to be accomplished, therapy had to completely suppress infection of all susceptible $CD4^+$ cells and that no additional reservoirs with longer decay characteristics could exist. These 2 conditions have proven difficult to achieve.

Chun et al. [6] described the presence of a small pool of $CD4^+$ T cells that had the resting-memory phenotype ($CD45RO^+$, $HLA\text{-}DR^-$) and that harbored infectious HIV-1 in untreated subjects [6]. Subsequently, this group and 2 others [7–9] described the persistence of this small reservoir, numbering, on average, 10^5–10^6 cells, despite prolonged and apparently suppressive antiviral therapy. The decay characteristics of this pool, although not precisely measured, are clearly slow [10, 11]. Furthermore, with the use of novel molecular assays and intensive

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Table 1. Characteristics of vaccinated and unvaccinated subjects in study of the effects of highly active antiretroviral therapy (HAART).

Group, subject	Time from symptoms to treatment, days	Baseline measurements		Treatment variables			Measurements at discontinuation of HAART					
		HIV-1 RNA, $\log_{10}$ copies/mL	$CD4^+$ T cell count, cells/mm^3	HAART regimen[b]	Duration of HAART, days	Δ $CD4^+$ T cell count, cells/mm^3	Stimulation index[c]	$CD8^+$ cell producing IFN-γ^+[a], %				
								Env	Gag	Pol	Pol/Nef	Total
1												
313-2	90	3.9	564	A	1822	+250	1.0	BD	BD	BD	BD	BD
1306	30	5.3	546	B	1105	+138	9.0	BD	0.13	BD	BD	0.13
1308	9	6.2	432	B	1209	+376	1.0	BD	BD	BD	BD	BD
1309	90	4.2	500	B	997	+234	2.0	BD	BD	0.18	0.19	0.37
1310	120	4.0	387	B	1210	+287	21.0	BD	0.34	BD	BD	0.34
2001	60	4.6	290	C	1470	+324	1.0	BD	0.10	BD	0.13	0.23
3002	60	5.0	459	C	1186	+758	4.0	BD	BD	BD	BD	BD
900	120	4.1	609	D	1175	+206	1.0	0.64	0.70	BD	0.98	2.32
904	60	4.4	689	D	1260	−19	4.0	BD	0.28	BD	BD	0.28
905	60	5.1	954	D	1115	+236	5.0	BD	0.12	BD	BD	0.12
918	9	6.2	473	D	1050	+202	8.0	BD	BD	0.12	0.10	0.22
Mean ± SD	64 ± 38	4.8 ± 0.7	536 ± 176	—	1236 ± 225	272 ± 291	5.2 ± 6.0	—	—	—	—	0.38 ± 0.7
2												
313-9	75	3.8	916	A	1042	+63	15.5	BD	BD	BD	0.08	0.08
1302	45	4.7	544	B	931	+432	6.3	0.21	BD	0.10	BD	0.31
1304	7	6.8	227	B	1182	+195	6.6	BD	BD	BD	BD	BD
1311	60	4.9	433	B	1056	+873	7.7	0.10	0.20	0.77	0.16	1.23
917	90	3.5	582	D	964	−38	1.0	BD	0.13	BD	0.06	0.19
Mean ± SD	55 ± 32	4.7 ± 1.3	540 ± 251	—	1035 ± 97	305 ± 362	7.4 ± 5.2	—	—	—	—	0.37 ± 0.5
Overall mean ± SD	62 ± 36	4.8 ± 0.9	535 ± 195	—	1173 ± 212	283 ± 244	5.9 ± 5.7	—	—	—	—	0.37 ± 0.6

NOTE. Group 1, vaccinated; group 2, unvaccinated. HIV-1, human immunodeficiency virus type 1; IFN-γ, interferon-γ.

[a] BD, below level of detection.

[b] A, zidovudine, lamivudine, and ritonavir; B, zidovudine, lamivudine, and indinavir; C, zidovudine, lamivudine, ritonavir, and saquinavir; D, zidovudine, lamivudine, abacavir, and amprenavir.

[c] Proliferative response of lymphocytes to HIV-1 Gag.

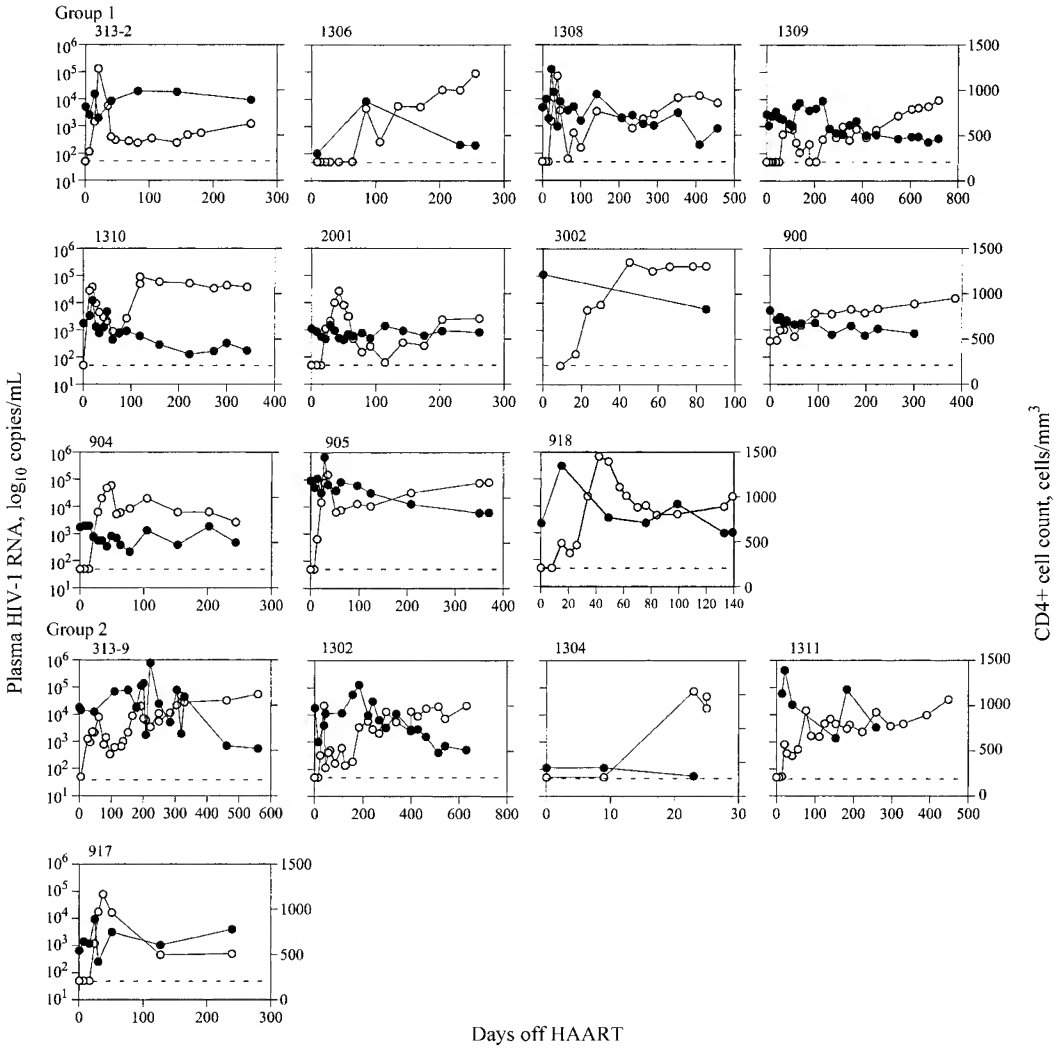

Figure 1. Longitudinal determinations of human immunodeficiency virus type 1 (HIV-1) RNA levels (○) and CD4 cell counts (●), in study subjects, either vaccinated (group 1) or unvaccinated (group 2), after highly active antiretroviral therapy (HAART) was discontinued.

genetic analyses, evidence has mounted that, despite apparently suppressive HAART, ongoing replication occurs in many subjects [12–17].

To date, discontinuation of HAART has been followed by a rapid virus rebound in plasma viremia [18–21]. Although these cohorts have been extremely heterogeneous, viruses in all subjects rebounded with similar kinetics, demonstrating mean initial plasma-viremia doubling times of ~2 days. All subjects in these cohorts were treated with HAART alone, and none received therapeutic immunization before termination of treatment.

Evidence in nonhuman primates, as well as that in humans, has suggested that simian immunodeficiency virus (SIV)– and HIV-1–specific immunity may be critical in the suppression of viral replication in vivo. Studies in the SIV macaque model by 2 groups have suggested that the early initiation of HAART, either when used with an adjunctive vaccine, NYVAC [22], or when given intermittently [23], may result in suppression of viremia when HAART is terminated. Furthermore, Rosenberg et al. [24] have reported that subjects treated very early during the HIV-1 primary-infection syndrome may suppress viremia to low levels when HAART is terminated either abruptly or intermittently.

Here we describe detailed virologic and immunologic characteristics of 16 subjects for whom HAART was initiated early during HIV-1 infection and who, after ~3 years, voluntarily discontinued treatment. Eleven subjects participated in a therapeutic vaccine trial of ALVAC vCP1452 and recombinant gp160 [25], whereas 5 subjects terminated treatment after HAART alone.

Table 2. Characteristics of vaccinated and unvaccinated subjects after highly active antiretroviral therapy (HAART) was discontinued.

Group, subject	Time to virus rebound, days	Initial plasma viremia measurements				Total time without therapy, days	Last Level of HIV-1 RNA[a], log_{10} copies/mL	Change in $CD4^+$ T cell count[a], cells/mm^3
		Doubling time, days	Peak, log_{10} copies/mL	Nadir after peak, log_{10} copies/mL	Change from peak to nadir, log_{10} copies/mL			
1								
313-2	6	1.4	5.2	2.4	2.8	259	3.1	76
1306	84	3.2	3.7	2.4	1.3	258[b]	5.0	−292
1308	16	1.4	4.9	1.8	3.1	456	3.9	−412
1309	68	4.5	2.9	1.7	1.2	708	4.0	−267
1310	13	1.4	4.6	2.9	1.7	342[b]	4.6	−301
2001	21	1.6	4.4	2.2	2.3	239	3.4	−42
3002	17	2.7	5.5	5.2	0.3	85[b]	5.4	−382
900	NA	5.7	3.3	2.8	0.5	380	4.2	−255
904	21	1.8	4.8	3.7	1.1	237	3.4	−170
905	14	1.6	5.2	3.8	1.4	371	4.9	−516
918	14	2.7	5.8	3.7	2.1	149	4.4	−79
Mean ± SD	27 ± 26	2.5 ± 1.4	4.6 ± 0.9	3.0 ± 1.0	1.6 ± 0.9	316 ± 167	4.2 ± 0.7	−196 ± 168
2								
313-9	26	4.5	3.9	2.5	1.4	559[b]	4.7	−459
1302	23	1.4	4.3	2.1	2.3	630[b]	4.3	−462
1304	23	1.7	4.7	NA	NA	23[b]	4.7	−123
1311	14	1.8	2.9	2.5	0.4	448[b]	4.6	−546
917	24	1.8	4.9	4.2	0.7	238	2.9	236
Mean ± SD	22 ± 5	2.2 ± 1.3	4.1 ± 0.8	2.9 ± 0.9	1.2 ± 0.9	379 ± 248	4.2 ± 0.8	−270 ± 326
Overall mean ± SD	26 ± 2	2.5 ± 1.4	4.4 ± 0.9	2.9 ± 1.0	1.5 ± 0.9	336 ± 189	4.2 ± 0.7	−213 ± 216

NOTE. Group 1, vaccinated; group 2, unvaccinated. HIV-1, human immunodeficiency virus type 1; NA, not applicable.

[a] Prior to reinitiation of treatment for some subjects.

[b] Prior to reinitiation of HAART.

Subjects, Materials, and Methods

Study subjects. Sixteen subjects were identified as newly HIV-1–infected, on the basis of the presence of plasma viremia associated with an absent or evolving antibody response to HIV-1 [3, 26, 27]. These subjects—15 men and 1 woman—had been infected via sexual contact, were symptomatic during primary HIV-1 infection, and began treatment 7–120 days after the onset of symptoms (table 1). Baseline HIV-1 RNA levels and $CD4^+$ T cell counts reflected the duration of infection, and study subjects were treated with 1 of 4 HAART regimens (table 1). Group 1 consisted of 11 subjects who participated in an adjunctive vaccine study as described by Jin et al. [25] and who elected to discontinue therapy after completion of the vaccination study; group 2 consisted of 5 HAART-treated unvaccinated subjects who elected to discontinue therapy. Inclusion criteria for discontinuation of drug therapy, for vaccination, or for both required participants to have sustained levels of plasma HIV-1 RNA below the detection level, 50 copies/mL (<1 detectable HIV-1 RNA determination per treatment year). In the 11 vaccinated subjects, HAART was terminated ~7 days after the last vaccination of 7 subjects, 14 days after vaccination of 2 subjects (subjects 1306 and 905), and ≥60 days after vaccination of the remaining 2 subjects (subjects 313-2 and 2001). After treatment had been discontinued, subjects were seen at weekly intervals for 4–8 weeks and subsequently as necessary.

Virologic determinations. Longitudinal plasma HIV-1 RNA levels were measured by use of a reverse-transcription polymerase chain reaction (PCR) (Amplicor HIV-1 Monitor Ultra Sensitive; Roche Molecular Systems) that has 50 HIV-1 RNA copies/mL as the lower limit of detection. Linear regression analysis assuming first-order kinetics was used to calculate initial doubling times of virus rebound in plasma. Genotypic analyses of patient plasma–derived *pol* sequences were performed by previously published methods [28], as well as by the TRUGENE HIV-1 Genotyping Assay with the Open Gene automated DNA sequencing system (Visible Genetics). Genotypic characterization of the CCR5 32-base (CCR5-Δ32) deletion was performed by polymerase chain reaction (PCR) of patient DNA isolated from peripheral-blood mononuclear cells. PCR amplification conditions were as previously published [29], and oligonucleotide primers flanking the CCR5-Δ32 deletion were used. Amplicons were analyzed on a 4% agarose gel.

Assay of the proliferation response of lymphocytes. The proliferative responses of peripheral-blood mononuclear cells were measured by a standard [^{3}H]thymidine (DuPont NEN) incorporation assay as described elsewhere [30]. The concentration of $p24_{NY5}$ (Protein Sciences) used for stimulation was 5 μg/mL.

Intracellular cytokine staining. $CD8^+$ T cell responses were measured as described elsewhere [25]. Aliquots of $0.5–1 \times 10^6$ cryopreserved peripheral-blood mononuclear cells from patients were infected with recombinant vaccinia viruses expressing HIV-1 antigens Env, Gag, Pol, and Pol/Nef or control antigen Eco (Virogenetics), at an MOI of 2.0 for 18–20 h at 37°C. Brefeldin A (10 μg/mL [Golgiplug; PharMingen]) was added during the last 5 h of incubation. The cells were stained with anti–CD3-phycoerythrin, anti–CD4-allophycocyanin, and anti–CD8/peridinin chlorophyll protein (Becton Dickinson) antibodies for 30 min at 4°C. After being washed, cells were permeabilized with CytoFix/Cytoperm solution (PharMingen) and then were stained intracellularly by an anti–interferon-γ (IFN-γ)/fluorescein isothiocyanate antibody (PharMingen) and were analyzed

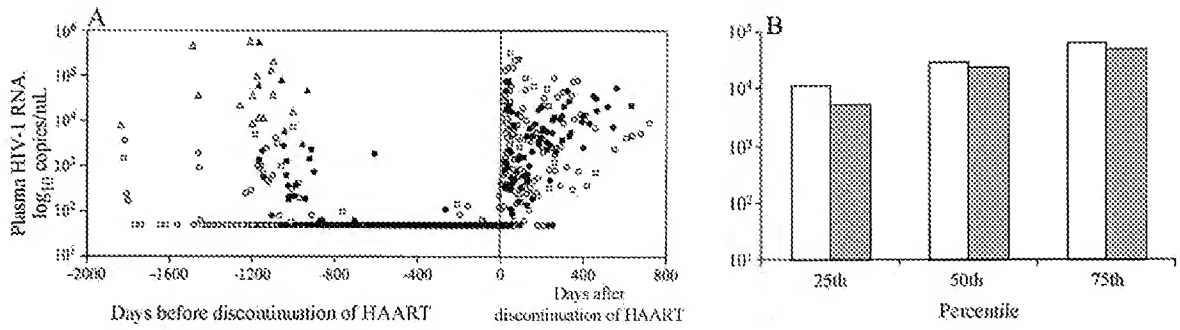

Figure 2. *A,* Distribution of human immunodeficiency virus type 1 (HIV-1) RNA levels before treatment (△, vaccinated; ▲, unvaccinated), during highly active antiretroviral therapy (HAART) *(left)* and after HAART was discontinued *(right)* (○, vaccinated; ●, unvaccinated). *B,* Distribution of HIV-1 RNA levels in untreated newly infected subjects after 1 year [31] *(white bars)* and in study subjects 222 days, on average, after HAART was discontinued *(gray bars).*

by a FACScalibur flow cytometer. The fluorescence-activated cell-sorter data were analyzed by CellQuest software (Becton Dickinson). The results were expressed as percentage of $CD8^+$ T cells producing IFN-γ. In the initial 195 consecutive assays for 43 HIV-1–infected subjects, the negative-control antigen Eco stimulated a mean (± SD) of 0.02% (±0.03%) of $CD8^+$ T cells to produce IFN-γ, whereas a 5-μg/mL concentration of positive-control superantigen, staphylococcal enterotoxin B, stimulated 1%–20% of the $CD8^+$ T cells to produce IFN-γ. On the basis of these preliminary experiments, 0.05% of IFN-γ–producing cells were found to be significantly above the background level and were considered to as having a positive value.

Statistical determinations. Statistical analyses included paired and unpaired Student's *t* tests, Mann-Whitney test, and simple regressions and were performed by StatView (SAS Institute).

Results

Study subjects. A total of 16 subjects, 11 vaccinated and 5 treated with HAART alone, discontinued therapy after a mean of 1169 days (range, 931–1822 days). The duration of treatment with HAART was slightly longer for vaccinated subjects (mean, 1230 days vs. 1035 days) and approached statistical significance ($P = .087$). All but 1 subject (subject 900) were considered, on the basis of interview and drug reconciliation, to have adhered to therapy. It is noteworthy that, of the 16 subjects, 5 (subjects 313-2, 1308, 904, 1304, and 917) were found to be heterozygous for the CCR5-Δ32 deletion.

Virologic measurements after discontinuation of drug therapy. At the time when therapy was discontinued, all subjects except subject 900 had plasma HIV-1 RNA levels of <50 copies/mL. Subject 900 admitted to nonadherence at the end of the treatment period and discontinued therapy without the knowledge of the investigators. All subjects experienced virus rebound after treatment was discontinued (figure 1), with viremia detectable after a mean of 26 days (range, 6–84 days) (table 2).

A mean initial peak plasma viremia of 4.4 $\log_{10}$ copies/mL (range, 2.9–5.8 $\log_{10}$ copies/mL) was reached after a mean of 41 days (range, 19–95 days). This was followed by a spontaneous, transient, and significant ($P < .001$) reduction—a mean of 1.5 $\log_{10}$ copies/mL (range, 0.3–3.1 $\log_{10}$ copies/mL)—in 15 subjects after treatment was discontinued (subject 1304 was excluded because of reinitiation of treatment). After a mean of 336 days of observation (range, 23–708 days), no subjects had maintained HIV-1 RNA levels that were <500 copies/mL. Of the 16 subjects, 4 (subjects 313-2, 2001, 904, and 917) had sustained levels of plasma virus that were <5000 copies/mL (3.7 $\log_{10}$ copies/mL) (table 2). Interestingly, 3 of these 4 subjects were heterozygous for the CCR5-Δ32 deletion. Of the remaining 12 subjects, 7 restarted HAART after a mean of 336 days (range, 23–630 days) at mean plasma HIV-1 RNA levels of 4.8 $\log_{10}$ copies/mL (range, 4.3–5.4 $\log_{10}$ copies/mL) and after an associated drop, in $CD4^+$ T cell counts, of 366 cells/mm^3 (table 2). The remaining 5 subjects maintained HIV-1 RNA levels of 3.9–5.0 $\log_{10}$ copies/mL.

When the cumulative virologic data for the entire cohort are examined (figure 2*A*), certain patterns become apparent. HAART resulted in a tapering of the distribution of HIV-1 RNA measurements over time, leading to coalescence toward the line corresponding to a virus load below the level of detection, here considered to be 50 copies/mL. This level was maintained in nearly all of the subjects until drug therapy was discontinued, which was followed by a rapid increase in the distribution of HIV-1 RNA levels, independent of vaccination status. Although this initial increase in viremia was typically followed by a spontaneous decrease, it was not sustained, as shown by a gradual widening in the distribution of HIV-1 RNA levels over time. In all 16 subjects, the distribution of HIV-1 RNA levels as last observed after HAART was discontinued was nearly identical to the distribution that Lyles et al. [31] reported for plasma viremia in 151 untreated subjects after 1 year of new HIV-1 infection (figure 2*B*). When comparing the characteristics of virus rebound in vaccinated subjects versus subjects treated with HAART alone, we could not identify significant differences in the time to virus rebound ($P = .22$), the initial rate of virus rebound ($P = .95$), peak viremia ($P = .28$)

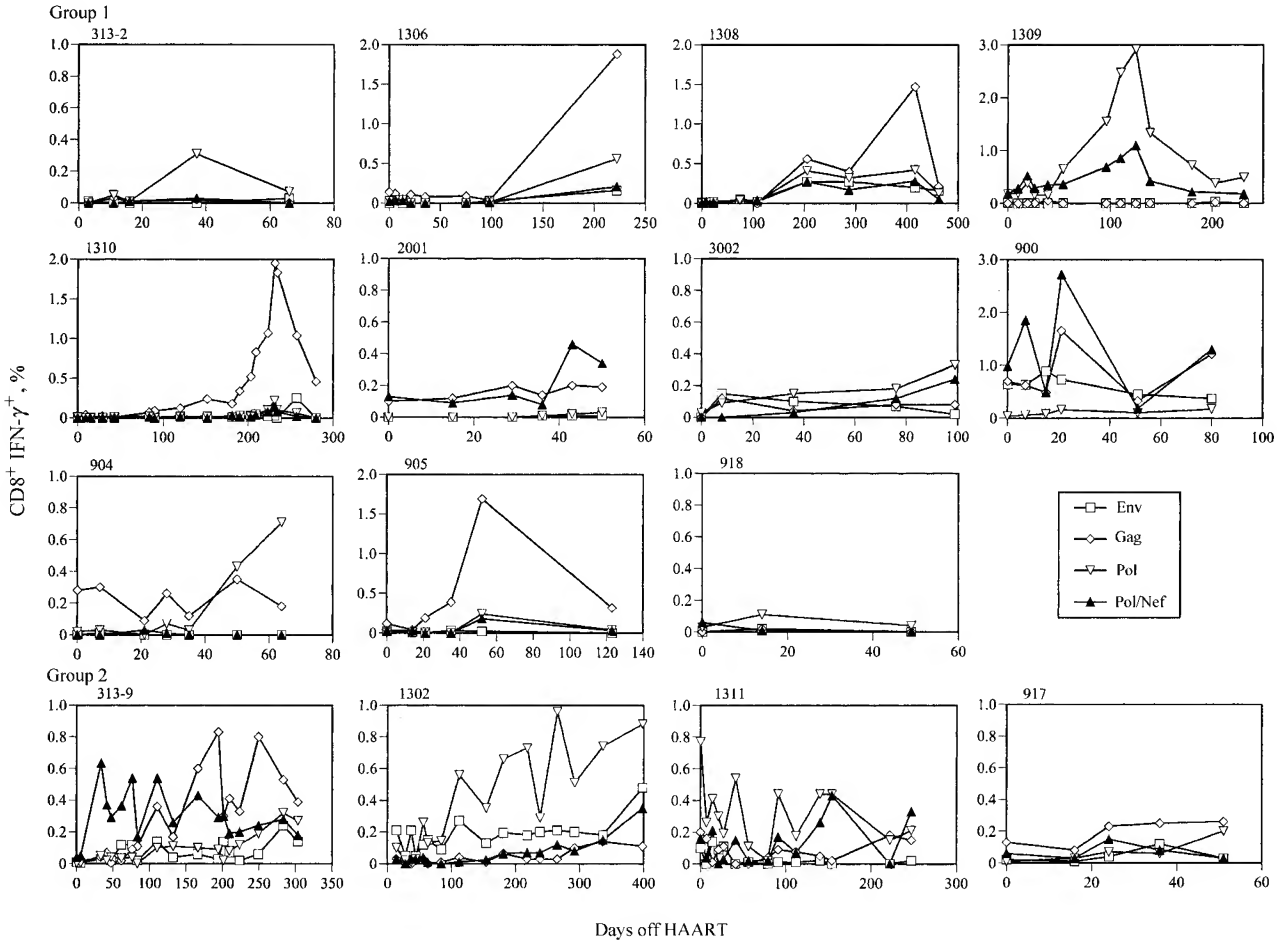

Figure 3. Longitudinal assessments of $CD8^+$ T cell human immunodeficiency virus type 1 (HIV-1)–specific immune responses after highly active antiretroviral therapy (HAART) was discontinued, in vaccinated (group 1) and unvaccinated (group 2) subjects. Results are expressed as percentage of $CD8^+$ T cells producing interferon-γ (IFN-γ) in response to HIV-1 antigens Env, Gag, Pol, and Pol/Nef.

after treatment had been discontinued, and HIV-1 RNA level as last observed after therapy was discontinued ($P = .65$).

Populations with virus rebound were studied to determine susceptibility to antiretroviral agents. Consensus genotypic analyses of the *pol* region of baseline and rebounding viruses from the plasma of 15 of 16 subjects (adequate material was not available for subject 918) did not reveal the emergence of viruses harboring primary resistance–conferring amino acid substitutions after HAART was discontinued (data not shown). Follow-up data are available for 6 subjects who required reinitiation of therapy. All responded well to a variety of combination antiretroviral regimens, although, because of lack of drug availability, subject 1311 subsequently stopped therapy a second time (data not shown). The remaining subject (subject 1306) who required reinitiation of therapy was lost to follow-up shortly after treatment reinitiation was advised.

Immunologic measurements after discontinuation of drug therapy. We studied HIV-1–specific cell-mediated immunity longitudinally. Total levels of $CD8^+$ T cell–mediated immunity, as well as individual responses to Env, Gag, Pol, and Pol/Nef, when drug therapy was discontinued are shown in table 1. We could not correlate differences in the dynamics or magnitude of virus rebound with either the quantity or quality of the $CD4^+$ T cell– or $CD8^+$ T cell–mediated immune responses of vaccinated, unvaccinated, or all subjects.

Longitudinal profiles of both $CD4^+$ T cell– and $CD8^+$ T cell–mediated HIV-1–specific immunity were determined after treatment had been discontinued (figures 3 and 4). Despite measurable increase of one or both cellular immune responses during the observation period, no consistent relationship between the level of these immune responses and the subsequent virologic profiles could be demonstrated.

In subject 1309, who had delayed virus rebound and a substantially longer plasma-viremia doubling time, initial suppression was associated with a substantial early increase in $CD8^+$ T cell responses (figure 3). Similarly, subject 900, inter-

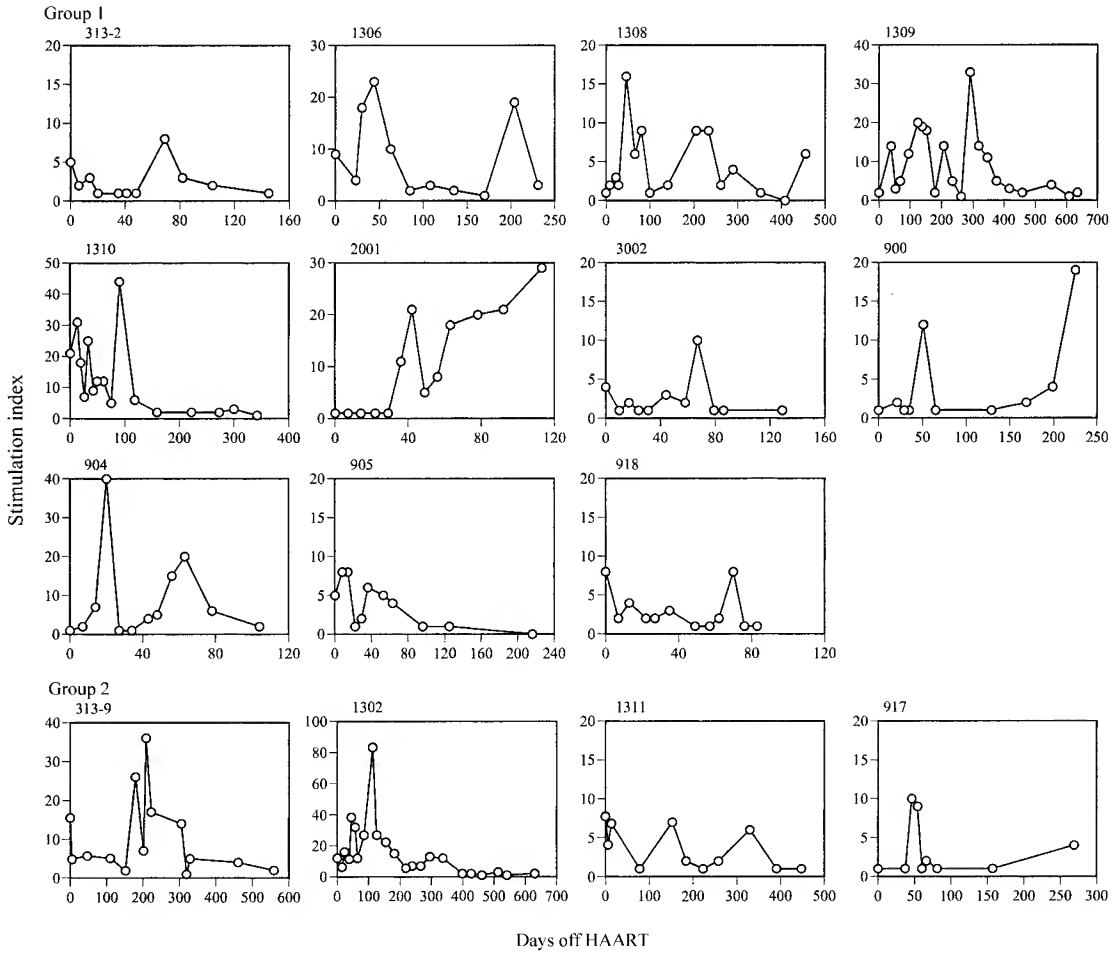

Figure 4. Longitudinal levels of the proliferative response of lymphocytes to the Gag antigen of human immunodeficiency virus type 1, expressed as stimulation indices in vaccinated (group 1) and unvaccinated (group 2) subjects, after highly active antiretroviral therapy (HAART) was discontinued.

mittently viremic because of nonadherence to the treatment regimen, had the most robust $CD8^+$ T cell responses (figure 3) and the longest initial plasma-viremia doubling time. However, in subject 1306, initial virus rebound was very delayed and was associated with both a dramatic increase in the proliferative response of lymphocytes to Gag (figure 4) and a monospecific $CD8^+$ T cell response (figure 3).

Viremia appeared to stimulate HIV-1–specific cellular immune responses in most subjects. Early increases in $CD8^+$ T cell responses temporally associated with initial virus rebound were observed in subjects 313-2, 1309, 2001, 3002, 900, 904, 918, 313-9, 1302, 1311, and 917 (figure 3). Similarly, substantial increases in the proliferative response of lymphocytes to Gag, as defined by an increase in the stimulation index to >10, accompanied initial virus rebound in subjects 1306, 1308, 1309, 1310, 2001, 900, 904, 313-9, and 1302 (figure 4). These $CD4^+$ T cell– and $CD8^+$ T cell–mediated immune responses were quite variable in depth and breadth and did not necessarily correspond temporally or quantitatively with subsequent suppression of viremia.

Subjects 2001 and 1302 exhibited increase, in both $CD4^+$ T cell and $CD8^+$ T cell responses, that corresponded temporally with a 2.3-log reduction in peak viremia. Subjects 1308 and 904 exhibited, respectively, 3.1- and 1.1-log reductions in viremia, a result temporally associated with an increase in only the proliferative response of lymphocytes to Gag.

Interestingly, subjects 313-2 and 918 experienced spontaneous 2.8-log and 2.1-log reductions, respectively, in HIV-1 RNA levels, reductions that were not associated with substantial measurable increases in either $CD4^+$ T cell– or $CD8^+$ T cell–mediated HIV-1–specific immunity. These data suggest either that the techniques used to detect HIV-1–specific immune responses in these subjects were inadequate or that as-yet-unidentified factors are at play here.

While subjects were receiving HAART, mean $CD4^+$ T cell counts increased by 272 cells/mm^3 (range, −38 to +873 cells/

mm^3) in the 16 subjects (table 1). On average, subjects lost 213 $CD4^+$ T cells/mm^3 during the period when treatment was discontinued (range, +236 to −462 cells/mm^3) (figure 1 and table 2). For the 12 subjects in whom plasma HIV-1 RNA levels rebounded and persisted at >5000 copies/mL, the $CD4^+$ T cell loss was more pronounced, 312 cells/mm^3, and exceeded the gain in absolute $CD4^+$ T cells that was achieved during HAART. It is noteworthy that $CD4^+$ T cell loss was generally gradual in the subjects, with the exception of subject 1304, who, within weeks after treatment had been discontinued, experienced an acute decrease in the level of $CD4^+$ T cells (figure 1).

Discussion

Early initiation of therapy during HIV-1 infection, with a combination of nucleoside reverse-transcriptase inhibitors and potent protease inhibitor(s), resulted in sustained suppression of plasma viremia to levels below detection in this cohort of 16 subjects. Despite prolonged HAART and apparent suppression of viral replication with or without adjunctive therapeutic vaccination, all subjects experienced virus rebound when treatment was discontinued. With few exceptions, the dynamics of virus rebound were rapid and not significantly different from those observed in cohorts of chronically infected subjects who had discontinued therapy [18–21]. We did not find significant differences, in the immediate post-HAART–discontinuation profiles, between the vaccinated subjects and those treated with HAART alone.

Of the 16 subjects who did not resume therapy, 4 suppressed plasma viremia to <5000 copies/mL; 3 of these 4 were treated with both HAART and vaccination, and 1 was treated with HAART alone. It is noteworthy that the distribution of HIV-1 RNA levels ~1 year after drug therapy had been discontinued in our cohort is nearly identical to that observed in an untreated cohort of newly infected subjects followed during the pre-HAART era. This clearly raises the question as to whether the virologic set point is altered by early intervention.

Recently published studies have suggested that the virologic set point can be altered by early interventions. Hel et al. [22] treated SIV-infected macaques with HAART and NYVAC-SIV-*gag-pol-env,* a highly attenuated recombinant pox vaccine. HAART was begun 14 days after infection and was discontinued 4 weeks after the third vaccination. Control groups received treatment with either HAART alone or vaccine alone. Although the study was limited by an assay with a lower limit of detection, 5000 SIV RNA copies/mL, viremia was suppressed in 4 of 7 macaques in the HAART-only group, as it was in 5 of 8 in the HAART-plus-vaccine group. Lori et al. [23] have recently shown that HAART given intermittently after 6 weeks of SIV infection is effective in suppressing SIV viremia in macaques after termination of antiretroviral therapy. Macaques treated either continuously or not at all fared poorly.

Rosenberg et al. [24] have recently described suppression of viremia to <500 HIV-1 copies/mL in 5 of 8 newly HIV-1–infected subjects undergoing intermittent interruption of treatment. This suppression was achieved after a single interruption of treatment ($n = 3$) or a second interruption of treatment ($n = 2$), in subjects followed up for a median of 6.5 months after therapy had been discontinued. The subjects in that study had much higher baseline HIV-1 RNA levels (6.7 $\log_{10}$ copies/mL vs. 4.8 $\log_{10}$ copies/mL), were nearly all preseroconversion at the time of treatment initiation, and were selected for study on the basis of measurements of the proliferative response of $CD4^+$ T cells to Gag. Only 3 of our subjects were treated before seroconversion (antibody-negative or -indeterminate), and their course could not be distinguished from that of the remaining 13 subjects. It is conceivable that the interval between infection and the initiation of HAART may account for the difference between the 2 experiences. Furthermore, our patients received more drugs (mean, 3.4 vs. 3.0 drugs) longer (3.2 years vs. 1.7 years) and have been followed longer after therapy has been discontinued. The relationship between the duration and intensity of HAART and the characteristics of virus rebound after therapy has been discontinued has not been established. However, taken together, the results of the studies by Hel et al. [22], Lori et al. [23], and Rosenberg et al. [24] suggest that perhaps shorter courses of therapy, initiated earlier and combined early with immunologic manipulations, may result in a more desirable outcome.

We did observe immunologic consequences when treatment was discontinued. In subjects who failed to suppress plasma viremia to <5000 copies/mL, the immunologic benefits of HAART achieved over 3.2 years were lost, on average, <1 year after drug therapy had been discontinued. It is noteworthy, however, that in no subject did the level of $CD4^+$ T cells decrease to <200 cells/mm^3. Furthermore, no clinical events associated with HIV-1 infection were seen during this period.

Of the 4 subjects with levels of HIV-1 RNA that were <5000 copies/mL of plasma after interruption of treatment, 3 were found to be heterozygous for the CCR5-Δ32 deletion [32]. This genotype is known to be associated with lower HIV-1 RNA levels in plasma 9–18 months after seroconversion [29]. Interestingly, 5 of the 16 subjects who elected to discontinue drug therapy were found to be heterozygous for this genetic polymorphism. This prevalence is appreciably higher than that observed among subjects newly infected with HIV-1 [29] and perhaps results from a selection bias, in that our subjects were chosen on the basis of levels of HIV-1 RNA that, during HAART, were persistently below the level of detection. It has been suggested that heterozygotes for the CCR5-Δ32 deletion may have a superior response to antiretroviral therapy [33]. Alternatively, since the cohort in the present study is exclusively white, a higher-than-expected prevalence of this genetic polymorphism is likely. For this reason, comparisons of this cohort with untreated random cohorts may be problematic. Nevertheless, the distribution of the last observed HIV-1 RNA level

after HAART has been discontinued in this cohort is not significantly different from that of HIV-1–infected subjects after 1 year of untreated infection [31].

Despite the seemingly negative findings presented above, there are critically important positive findings that merit discussion and support continued aggressive approaches to treatment of primary and early HIV-1 infection in the research setting. The results reported by Rosenberg et al. [24], Hel et al. [22], and Lori et al. [23] suggest that the host immune system is able to modify virus rebound when therapy is discontinued. In the present study, we did observe that slower plasma-viremia doubling times, modest peak levels of viremia, and short-lived spontaneous declines in plasma HIV-1 RNA levels are all consistent with partial immune suppression of viral replication in the absence of drug therapy. However, the induced immune responses appeared to have only a transient effect on HIV-1 replication in vivo in the majority of subjects. Furthermore, the immune responses that were measured were extremely variable, and no clear correlate of initial virologic suppression could be established.

It should be noted that the measurements of HIV-1–specific immunity that have been used in the present study are limited. Levels of $CD8^+$ T cell activity against HIV-1–specific antigens were confined to HIV-1 antigens Env, Gag, Pol, and Nef. It is now appreciated that immune responses to other HIV-1 antigens, such as Tat and Rev [34], may be of significance in the dissection of the cellular immune response to HIV-1. Furthermore, because of obvious constraints, the stimulating antigens used are based on laboratory strains and not on autologous patient virus. We accept the fact that the immune parameters that we are measuring are, at best, minimum estimates. We must also concede that the proliferative response of lymphocytes to Gag, although considered, at the time of our study, to be the optimal way to measure $CD4^+$ T cell–mediated HIV-1–specific immune function, has been replaced by newer, more convenient, and versatile assays, which likely provide more information than does the limited response to 1 antigen [35]. Our studies of the immune responses after treatment had been discontinued were limited to the cellular immune system. Indeed, additional studies, looking at the role of antibodies in the initial suppression of viremia, are indicated. We believe that further intensive study of these subjects, particularly those who appear to suppress viremia in the absence of detectable immune responses, is clearly indicated and is in progress.

That virus rebound was observed in our patients when treatment termination was discontinued indicates that the HAART regimens that we used were unable to reduce the total-body virus burden to a level that is controllable by an intact, measurable, and inducible host cellular immune response to HIV-1. It is our hope that further optimization of antiretroviral therapy can reduce the HIV-1 burden to a level that would be more amenable to immune control. We also believe that further investigations of immune-based therapies to augment HIV-1–specific responses are needed as an adjunct to optimized HAART, to facilitate durable suppression of HIV-1 replication after antiretroviral therapy has been discontinued. Given the long-term toxicities of HAART [36–39] and the cost of lifelong antiretroviral therapy, it is critical to develop treatment strategies that will effectively limit drug exposure while minimizing the impact that HIV-1 infection has on the host. We believe that such a goal should be given high priority in future clinical investigations and that early and aggressive treatment of primary HIV-1 infection must remain an area of intense study.

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